AMERICAN
JOURNAL OF BOTANY

VOL. II FEBRUARY, 1915 NO. 2

SOME EFFECTS OF THE BROWN-ROT FUNGUS UPON THE COMPOSITION OF THE PEACH[1]

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The morphology and life-history of *Sclerotinia cinerea* (Bon.) Schröter, the fungus causing brown-rot of the peach, have been investigated with considerable care. The fact that this fungus is parasitic upon a number of other hosts is also well established. Not so much attention, however, has been paid to the effect of the fungus upon the composition of the peach; and it was to obtain some information upon this subject that the experiments described in this paper were carried out.[2]

In these experiments most of the peaches were inoculated in the laboratory, though one series of analyses was made using peaches which had become infected under orchard conditions. The inoculations were made from stock cultures of the fungus grown on potato agar. The fungus was originally isolated from fruit in the early stages of brown-rot by removing portions of the deeper-lying tissue and transferring them to tubes of sterile potato agar. Pure cultures were obtained by this method and the fungus maintained in stock culture throughout the investigation.

The method for studying the effect of the fungus upon the tissue of the peach was somewhat similar to that used by Behrens (1) in

[1] From the Bureau of Plant Industry, U. S. Department of Agriculture, Office of Plant Physiological and Fermentation Investigations. Published by the permission of the Secretary of Agriculture.

[2] The writer's thanks are due to Mr. J. W. Kelly, laboratory technician in this office, for assistance in the experimental part of this work.

[The **Journal** for January (2: 1–70) was issued 18 Feb., 1915.]

studying the effects of *Sclerotinia fructigena* (Pers.) Nort. upon apples. This writer made a single inoculation upon the side of each apple. When the fruit was half rotted he split the rotten half from the sound portion and analyzed the two parts separately.

In the present investigation this method was considerably modified. The peaches were first divided into quarters and the opposite quarters were combined. One of the two samples thus obtained was inoculated and the other was used as a control. Before the peaches were sampled, however, they were thoroughly scrubbed with a solution of bichlorid of mercury 1 to 1,000. They were quartered and separated from the stone with a sterile knife and immediately placed in sterile, glass-stoppered weighing bottles which had been tared, and then weighed. One set of these samples consisting of one sample from each peach was inoculated from the stock cultures of the fungus by shaking up spores in sterile water and pouring them over the cut surfaces of the fruit. The other set, the controls, were treated with a like quantity of sterile water. The two sets of samples were kept side by side under the same temperature and moisture conditions for two weeks or more. At the end of this time the samples were examined and any of the control samples found to be infected with fungi were discarded together with the corresponding inoculated halves. This procedure was also followed with any of the inoculated portions found to be infected with fungi other than Sclerotinia. As a further precaution cultures on beef agar were made from the interior of the remaining samples and if any of these cultures indicated the presence of contaminating fungi or bacteria in the fruit the samples were discarded. After these inoculations were made the samples were prepared for analysis

In the analyses, determinations were made of the pentosan, acid, and sugar content of the peach samples. The amount of alcohol-insoluble material which reduced Fehling's solution when hydrolyzed with dilute hydrochloric acid, was also determined. The data thus obtained were calculated on the basis of wet weight of fruit at the time of inoculation and it was then possible to compare the content of the different compounds in the sound and rotten portions of the same peach.

In the pentosan and acid determinations it was found advisable to use an entire sample for each determination. In the case of the sugars, however, a sample served for the two sugar determinations and for the determination of alcohol-insoluble substance which reduced

Fehling's solution upon hydrolysis with dilute hydrochloric acid. The samples were prepared for analysis by slicing as thinly as possible with a sharp knife and then chopping up the slices. Inasmuch as all determinations were related to original wet weight, care was taken that none of the pulp or juice was lost.

For pentosan determinations the chopped-up peach pulp was washed directly into half liter Erlenmeyer flasks and distilled immediately. Pentosan determinations were made according to Tollens's phloroglucid method (8) and the phloroglucid was calculated as pentosan according to Krober's (8) tables. Methyl-pentosan determinations made on portions of a number of peaches according to Ellett and Tollens's (9) method, showed the presence in the peach-pulp of substances yielding methyl-furfurol when boiled with hydrochloric acid. The amount, however, was so small that it was not considered worth while to determine it in all the samples.

The samples for acid determination were washed into 250 cc. Erlenmeyer flasks with 100 cc. of water and heated nearly to boiling for one hour. The mixture was then transferred to 250 cc. volumetric flasks which were filled to volume with water and allowed to stand with frequent shaking for one week. Toluol was added to prevent the action of micro-organisms. At the end of a week the solutions in the flasks were again made up to volume, filtered, and two 50 cc. portions of the filtrate titrated against standard potassium hydroxide. The acidity of the samples was calculated from these data in cc. normal acid per 100 g. original wet weight.

The samples in which the sugar determinations were made were washed into 250 cc. volumetric flasks with 70 per cent alcohol. About a gram of calcium carbonate was added to neutralize the acidity. The flasks were filled up to volume with alcohol and allowed to stand with frequent shakings for one week. At the end of this time the solutions were again made up to volume, filtered, and 200 cc. of the filtrate pipetted into beakers. The alcohol was driven off and the residue washed into volumetric flasks with water. The solution was cleared with neutral lead acetate, made up to the original volume and filtered. The excess lead was precipitated as oxalate, by adding sodium oxalate. The solution was again filtered and the amount of reducing sugar determined in the filtrate, using Allihn's modification of Fehling's solution (8). The copper was determined by direct weighing of the cuprous oxide and the dextrose calculated according to

Allihn's tables. The method used for sugars was similar to that given by Bryan, Given and Straughn (10).

The total sugars were determined in the solution used for reducing sugars by inverting the sucrose in 50 cc. with hydrochloric acid, making the solution up to 100 c.c. and neutralizing with anhydrous sodium carbonate. The sugar was determined as in the case of the reducing sugar. The sucrose content was calculated from the difference in total and invert sugar in the sample, as is the usual procedure in investigations of this kind. Kulisch (7), Girard (5), Bigelow and Gore (2) and others have considered this difference between the total and reducing sugars in peaches to be due to sucrose. It was, however, considered worth while, in view of the work of Davis and Daish (4), to obtain more evidence upon this point. Accordingly, a number of peaches were sliced up, a little calcium carbonate added, and the mixture extracted with 70 per cent alcohol, for one week. The solution was then filtered and three samples of the filtrate measured out. These solutions were prepared for analysis in the same way as the solutions from samples of peaches in the inoculation experiments and the reducing sugars determined. For total sugars duplicate samples were pipetted from each of the three solutions. The one set of these was treated with acid in the usual way, while the sugar in the other set was inverted with invertase.[3] The percentage of reducing sugar in the solutions and the percentage of total sugar as found by both methods are given below:

	Per Cent Sugar as Glucose after Inversion (Total Sugar)	
Per Cent Reducing Sugar	Acid Inversion	Invertase Inversion
0.77	3.34	3.37
0.77	3.36	3.48
0.77	3.47	3.46

From these results it would seem probable that the increase in reducing substance after treatment with acids is due to the inversion of the cane-sugar in the solution. At any rate, according to the work of Hudson (6), the increase in reducing power is not due to the hydrolysis of starch, dextrins or pentosans or to the inversion of maltose or lactose. The alcoholic sugar solution, from which the original samples were taken, was later cleared with lead acetate, filtered, and the excess lead removed as sulphide. The filtrate was evaporated and a sugar

[3] The writer's thanks are due Dr. C. S. Hudson, of the Bureau of Chemistry, for the invertase solution used in these experiments.

crystallized out. After several recrystallizations this sugar was identified as sucrose by its melting point, by the fact that it did not reduce Fehling's solution until after inversion with acid or invertase and by its specific rotation.

As has been said, the alcoholic sugar solution was filtered off the peach sample and the amount of sugar determined in the filtrate. The residue on the filter was carefully removed to a porcelain extraction thimble and extracted for one day continuously in a Soxhlet extractor. It was then dried, ground up and treated according to the usual routine for the determination of starch. The determinations were made by the direct acid hydrolysis method (8), as in the case of the sugars. Whether any considerable amount of these substances, which are insoluble in alcohol and reduce Fehling's solution after hydrolysis with dilute hydrochloric acid, are starch, is an open question. Bigelow and Gore (2) found starch grains in young peaches located only in a thin layer just under the epidermis. Determined quantitatively they found only one tenth of one per cent. The present writer examined portions of the ground pulp microscopically and, while bodies were present which gave the blue color with iodine, no definitely formed starch grains were discovered.

To obtain more evidence upon this point, six twenty-five gram samples of peach pulp were weighed out and extracted. After drying, three of the samples were treated by the direct acid hydrolysis method and the reducing substances determined in the usual way. The other three samples were carefully ground with fine sand and then digested with malt diastase and treated as in the diastase method for the determination of starch (8). The reducing substance was determined as in the other three samples. A comparison of the percentage of reducing substance in the two sets of samples, calculated as starch, is given below.

Acid Hydrolysis	Diastase Method
1.24	0.32
1.33	0.38
1.34	0.37

From the results of these determinations it seems that a portion of alcohol-insoluble substance which reduces Fehling's solution after hydrolysis with dilute hydrochloric acid, is either starch or some compound, such as dextrin, which is liquefied by diastase. The present work and the investigations of Bigelow and Gore would seem

to indicate that in the peach fruit starch is not a common form of reserve for carbohydrates translocated from other parts of the plant.

The method of studying the effect of the fungus upon the peach in this work was to compare the percentage of the compounds as determined in the two samples taken from the same peach, one of which had been inoculated with the fungus, while the other remained sound. Any considerable variation between the two halves in the percentage of the substances determined was considered to have been caused by the fungus. This method is based on the assumption that the content of any of the substances determined is the same in the two samples of the same peach. It seemed then, of some importance to determine the extent of the variation between the two halves of the peach sampled in this way. Accordingly, a series of experiments were carried out using sound peaches and following the same method of sampling as in the inoculation experiments, except that the halves were prepared for analysis immediately after sampling. The same variety of peaches, namely Champion, was used in these determinations as in the inoculation experiments described later.

The results of these analyses are given in Tables I and II, which follow:

TABLE I

PENTOSAN AND ACID CONTENT, AND THE AMOUNT OF ALCOHOL-INSOLUBLE SUBSTANCE WHICH REDUCES FEHLING'S SOLUTION WHEN HYDROLYZED WITH DILUTE HCl, IN SOUND PEACHES, EACH SUBSTANCE DETERMINED IN THE TWO HALVES OF THE SAME PEACH

	Per Cent of Pentosans, Wet Weight		Acid Content in cc. Normal Acid per 100 g., Wet Weight		Per Cent Alc.-Insol. Substance (as Starch) Reducing Fehling's Sol. when Hydrolyzed with Dil. HCl, Wet Weight	
	Half *a*	Half *b*	Half *a*	Half *b*	Half *a*	Half *b*
1	1.21	1.28	13.55	13.21	1.92	1.76
2	1.26	1.37	11.57	12.50	1.54	1.48
3	1.03	1.05	14.67	15.64	1.78	1.64
4	0.97	0.97			1.92	2.01
5					2.01	1.88

From the results in the foregoing tables it seems that there is occasionally some variation in the composition of the two samples. This variation, however, is not as great as between the individual peaches.

Two series of experiments were carried out in which the peaches were inoculated in the laboratory. In the first series of experiments

TABLE II

REDUCING SUGAR, TOTAL SUGAR, AND SUCROSE CONTENT IN THE HALVES OF THE SOUND PEACHES, STATED AS PERCENTAGE OF WET WEIGHT

	Reducing Sugar as Glucose		Total Sugar as Glucose		Sucrose	
	Half *a*	Half *b*	Half *a*	Half *b*	Half *a*	Half *b*
1	4.23	4.33	7.14	6.63	2.57	2.17
2	4.01	4.24	6.00	6.28	1.98	1.91
3	3.50	3.54	5.52	5.56	1.92	1.92

twenty peaches were picked, sampled and inoculated July 23. They were prepared and inoculated as already described and were allowed to remain in the glass-stoppered weighing bottles which were placed in a large covered glass dish until August 11. They were then examined and prepared for analysis.

The second series of experiments was set up August 14; the same number of peaches were used as in the first series and they were treated in the same way. The samples were prepared for analysis in the usual way August 30. The results of the determinations in the two series of inoculation experiments are given in tables 3–6, which follow:

TABLE III

PENTOSAN AND ACID CONTENT AND THE CONTENT OF ALCOHOL-INSOLUBLE SUBSTANCE WHICH REDUCES FEHLING'S SOLUTION WHEN HYDROLYZED WITH DILUTE HCl, IN THE SOUND AND ROTTEN HALVES OF PEACHES, EACH SUBSTANCE DETERMINED IN SOUND AND ROTTEN HALVES OF THE SAME PEACH

	Per Cent of Pentosans, Wet Weight		Acid Content in cc. Normal Acid per 100 g., Wet Weight		Per Cent Alc.-Insol. Substance (as Starch) Reducing Fehling's Sol. when Hydrolyzed with Dilute HCl, (Wet Weight)	
	Sound Half	Rotten Half	Sound Half	Rotten Half	Sound Half	Rotten Half
1	0.93	0.92	4.75	5.70	1.54	1.39
2	1.17	1.17	7.54	8.85	1.12	1.05
3	0.88	0.94	4.28	5.32	1.30	0.95

TABLE IV

REDUCING SUGAR, TOTAL SUGAR, AND SUCROSE CONTENT IN THE SOUND AND ROTTEN HALVES OF PEACHES, STATED AS PERCENTAGE OF WET WEIGHT

	Reducing Sugar, as Glucose		Total Sugar, as Glucose		Sucrose	
	Sound Half	Rotten Half	Sound Half	Rotten Half	Sound Half	Rotten Half
1	2.41	2.83	4.43	2.836	1.92	0.004
2	2.06	3.08	4.59	3.24	2.31	0.14

TABLE V

PENTOSAN AND ACID CONTENT, AND THE AMOUNT OF ALCOHOL-INSOLUBLE SUBSTANCE WHICH REDUCES FEHLING'S SOLUTION WHEN HYDROLYZED WITH DILUTE HCl, IN THE SOUND AND ROTTEN HALVES OF PEACHES. EACH SUBSTANCE DETERMINED IN SOUND AND ROTTEN HALVES OF THE SAME PEACH.

	Per Cent Pentosans Wet Weight		Acid Content in cc. Normal Acid per 100 g., Wet Weight		Per Cent Alc.-Insol. Substance (as Starch) Reducing Fehling's Sol. When Hydrolyzed With Dil. HCl, Wet Weight	
	Sound Peach	Rotten Peach	Sound Peach	Rotten Peach	Sound Peach	Rotten Peach
1	0.57	0.59	2.45	7.40	0.83	0.80
2	0.67	0.69	4.75	7.30	0.73	0.64
3	0.62	0.66	4.25	6.55	0.71	0.67
4					0.85	0.59

TABLE VI

REDUCING SUGAR, TOTAL SUGAR, AND SUCROSE CONTENT IN THE SOUND AND ROTTEN HALVES OF PEACHES, STATED AS PERCENTAGE OF WET WEIGHT.

	Reducing Sugar as Glucose		Total Sugar as Glucose		Sucrose	
	Sound Half	Rotten Half	Sound Half	Rotten Half	Sound Half	Rotten Half
1	2.47	4.14	5.95	4.26	3.31	0.12
2	1.35	1.68	3.93	1.88	2.44	0.19
3	1.14	2.63	3.03	2.82	1.79	0.18

A third series of analyses was begun August 16, using fruit that had become infected under orchard conditions. A quantity of peaches were picked, some of which bore typical brown-rot spots upon the surface. A number of the peaches which were apparently sound were selected, the pulp removed from the stones and cut up into thin slices. The whole mass was then chopped up into small pieces, well mixed and sixteen twenty-five gram samples weighed out, four for the determination of each of the various substances. In addition to these, three ten-gram samples were weighed out for the determination of the total dry matter. These last were covered with 95 per cent alcohol in glass-stoppered weighing bottles and the samples allowed to stand for several days. Most of the alcohol and water was then driven off at about 60° C. The residue was dried to constant weight in an oven at a temperature of 100° C. and the amount of dry matter calculated.

The infected peaches were allowed to remain under warm humid conditions for two days after the sound peaches were sampled. At the end of this time they were rotten and in most cases covered with tufts

of conidiophores of the fungus. They were sliced up and sampled and the samples treated as in the case of the sound peaches. The results of the determinations are given in Table VII, which follows.

TABLE VII

PENTOSAN, ACID AND SUGAR CONTENT AND AMOUNT OF ALCOHOL-INSOLUBLE SUBSTANCE WHICH REDUCES FEHLING'S SOLUTION ON HYDROLYSIS WITH DILUTE HCl, in SOUND AND ROTTEN PEACHES FROM SAME TREE

	Wet Weight		Dry Weight	
	Sound	Rotten	Sound	Rotten
Per cent dry matter	14.40	14.40		
Per cent reducing sugar	4.28	8.50	29.75	59.05
Per cent total sugar	9.32	8.59	64.69	59.65
Per cent sucrose	4.89	0.08	33.22	0.57
Per cent alc. insol. substance (as starch) which reduces Fehling's sol. when hydrolyzed with dil. HCl	1.30	1.07	9.05	7.44
Per cent pentosans	0.76	0.73	5.30	5.08
Cc. normal acid per 100 g	10.53	13.00	73.12	90.28

From the results in the foregoing tables it is evident that some of the compounds studied are much more readily available for the metabolism of the fungus than others.

The pentosan content of the sound and rotten halves of the peaches was usually about the same, the differences being no greater than the variations in pentosan content of the two halves of a sound peach. Moreover, the pentosan content was sometimes higher in the rotten half of the peach than in the corresponding sound half. It seems probable then, that the pentosans were not utilized by the fungus. Cooley (3) has recently shown that the hyphae of this fungus are not found to any considerable extent in the middle lamellae of the cells and it does not apparently digest the pectin of plum fruits. It is possible, though not probable, that part of the pentosan, or furfurol-yielding material, might be used by the fungus and a like amount of pentosans laid down.

The acid content was always higher in the rotten half of the peach than in the sound portion and this difference was greater than the variation in acid content between the two halves of a sound peach. It would seem then, that the fungus forms some acid or causes it to be formed by the peach. No attempt was made in the present investigation to identify the acids of either the sound or rotten peach. This work would, however, seem to corroborate the investigations

of Cooley (3) who found that oxalic acid was formed when this fungus was grown upon peach-juice. Behrens (1), on the other hand, found in his work with apples that the acid content of the rotten half of the apple was considerably less than that of the sound portion. In this case the fungus apparently used the acid.

The percentage of alcohol-insoluble substance which reduces Fehling's solution when hydrolyzed with dilute hydrochloric acid was somewhat less in all the rotten samples than in the corresponding sound halves. It is, therefore, to be concluded that the action of the fungus tends to decrease the amount of this substance in the peach, although the variation between the sound and rotten halves of the same peach is occasionally less than that found between the two halves of a sound peach, as shown in Table I, columns 6 and 7. The substance may be utilized by the fungus or changed so that it is soluble in alcohol. In the latter case it would have been extracted with the filtrate used in the determination of the sugars.

In sugar content the sound and rotten halves of the peaches varied considerably. There was more reducing sugar in the rotten halves than in the corresponding sound portions, while in the total sugar content the order was reversed. There was very little cane-sugar in the rotten portions, much less than in the sound samples. The fungus then uses the sugar and causes the inversion of the sucrose. That the sucrose is inverted much more rapidly than the invert sugar is used by the fungus is evident from the fact that the reducing sugar content is higher in the rotten samples. This is especially evident in the case of the peaches rotted after they had become infected under orchard conditions in which only a small amount of the sugar had been used yet nearly all the cane-sugar had been inverted. Behrens (1) found that *Sclerotinia fructigena* used the sugar in apples; he, however, measured only the total sugars.

In conclusion, it may be said that in peaches rotted by the brown-rot fungus, *Sclerotinia cinerea*, the pentosan content remains practically the same, the acid content is increased, the amount of alcohol-insoluble substance which reduces Fehling's solution when hydrolyzed with dilute hydrochloric acid decreases, the total sugar content decreases, while the cane-sugar practically disappears.

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